EXPERIMENT 1: Single cycle growth curves

References

1. Adams, M. H., Bacteriophages, Interscience Publishers, Inc., N. Y., 1959, Appendix, p. 473 ff.

2. Epstein, R. H. et al., Physiological Studies of Conditional Lethal Mutants of Bacteriophage T4D, Cold Spring Harbor Symposium 28, 375, 1963.

3. Stent, G. S., Molecular Biology of Bacterial Viruses, W. H. Freeman and Co., San Francisco, 1963.

Bacteria

E. coli strains:

1. CR63 -- amber-permissive, allowing growth of T4 amber mutants
2. B -- amber non-permissive
3. K37 -- amber-permissive, Hfr, allowing growth of MS2 amber mutants
4. K38 -- amber non-permissive, Hfr. Parent of K37
5. C3000-- amber, non-permissive, Hfr

Bacteriophage

1. T4D and amber mutants
2. MS2 and amber mutants

Media

For MS2:

Bacto-tryptone	10 gm
Yeast extract	1 gm
Sodium chloride	8 gm
Glucose	1 gm
Calcium chloride	0.002 M
Distilled water	1 liter

For T4:

Bacto nutrient broth	8 gm
Bacto peptone	5 gm
Sodium chloride	5 gm
Glucose	1 gm
Distilled water	1 liter

The purpose of this experiment is to compare the growth curves of T4 and MS2 and "early" and "late" amber mutants of these phages. Time curves of phage formation will be done in permissive hosts and infective centers and 60 minute values only will be determined in non-permissive hosts. Each pair of students will use a single phage type as follows:

Pair 1 - T4r in CR63 and B
2 - MS2 in K37 and K38
3 - T4amN122 in CR63 and B
4 - MS2am9 in K37 and K38
5 - T4amN120 in CR63 and B
6 - MS2am2 in K37 and K38

To be supplied:

1. CR63, B, K37, K38 at 2 x 10^8/ml
2. Phage suspensions at 2 x 10^8 pfu/ml
3. MS2 medium; T4 medium

Protocol for permissive hosts

-5' - Incubate 1 ml of host cells in 13 x 100 mm tubes at 37°
0' - Infect at m o i of 0.1
5' - Dilute to 10^{-2}, 10^{-3}, 10^{-4} with appropriate medium (T4 or MS2)

(All 10^{-2} dilution steps should be done by transferring 0.1 ml to 10 ml, and all 10^{-1} dilution steps by transferring 0.5 ml to 4.5 ml) Plate 0.1 ml of 10^{-2}, 10^{-3}, 10^{-4} dilutions immediately on permissive host.

Treat original culture with 1 drop of chloroform and continue incubating this tube (called the "original tube").

Continue incubating the 10^{-4} dilution only (called the "incubation tube").

10' - Remove 0.5 ml from the incubation tube into 13 x 100 mm tube. Add 1 drop chloroform and shake.

15' - Repeat sampling as at 10'.

20' - Repeat sampling
Also plate 0.1 ml from original tube after diluting to 10^{-2} and 10^{-3}.

25')
35')- Repeat sampling
45')

60' - Sample MS2 only

Handling of samples

To ~~MS2 samples only~~ all samples add 0.1 ml lysozyme-EDTA and incubate at 37° for 30'.

T4 samples - incubate at 37° for 30'.

Plate 0.1 ml of following final dilutions, using permissive host (you start with 10^{-4}):

10', 15' - 10^{-4}, 10^{-5}
20' - 10^{-5}, 10^{-6}
25' - T4: 10^{-5}, 10^{-6}; MS2: 10^{-5}, 10^{-6}, 10^{-7}
35', 45' - T4: 10^{-5}, 10^{-6}; MS2: 10^{-6}, 10^{-7}
60' - MS2: 10^{-7}, 10^{-8}

Protocol for non-permissive hosts

Same as for permissive hosts up to 10'

Always plate on permissive host!

15' - Plate 0.1 ml from original tube after diluting to 10^{-2} and 10^{-3}

60' - Remove 1 ml from incubation tube. Add 1 drop of chloroform and shake. ~~For T4: after incubating at 37° for 30 minutes, plate 0.1 ml.~~ For MS2+T4 treat with chloroform and lysozyme-EDTA as described above and plate 0.1 ml of 10^{-4}, 10^{-5}, 10^{-6} dilutions for MS2 + 10^{-4} for T4.

Next time:

Separate protocols for MS2 + T4

Have Marie melt soft agar + add supplements

Add supplements to broth